By facsimile + Confirmation by registered mail EUROPEAN PATENT OFFICE D-80298 MUNCHEN GERMANY

Your ref.: EP04789648.5-2405

Our ref.: B06788 CA/CS Lyon, 9<sup>th</sup> February 2009

Re.: European patent application n° 04789648.5- filed on October 27, 2004

IN THE NAME OF COMMONWEALTH SCIENTIFIC & INDUSTRIAL RESEARCH

ORGANISATION

RESPONSE TO OFFICIAL ACTION PURSUANT TO ARTICLE 94(3) EPC

DATED 29<sup>TH</sup> JULY 2008

Dear Sirs,

In reference to the official communication dated 29<sup>th</sup> July 2008, regarding the above-mentioned European patent application, we submit below a reply and a new set of claims, comprising 34 claims, which replaces the claims presently on file.

## I- Claim amendment and added subject-matter:

The claims presently on file have been amended in response to the objections raised by the Examining Division. Please replace pending claims by new claims 1-34 annexed to the present response.

Please find enclosed, in Annex A, a table detailing support in the application as filed for the amended claims.

Concerning subject matter of cancelled embodiments, Applicant reserves the right to file a divisional application to pursue its examination.

These amendments do not contravene the requirements of Art. 123(2) EPC.

# II- Novelty of the claims (Article 54 EPC):

In the official communication, the Examiner expresses the view that the claims to rice starch granules and to products comprising rice flour are not novel over the disclosure of document **D6** (WO02/101059) and **D7** (WO02/37955) newly introduced by the Examiner.

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P = 1 V= TS

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- [#7,846])) ( ] - 31-13 - 13 - [8](0])(13

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Magali CLAIR-MOULY, CPI Conseil européen en marques OHMI/OHIM This view is based on the assumption that high-amylose starch granules from rice cannot be differentiated from high-amylose starch granules from other plants. The Applicant disagrees.

In this respect, it is reiterated that, contrary to the Examiner's view, starch granules from different plants can be differentiated. Starch granules contain not only starch but also <u>proteins</u> and <u>lipids</u> which are characteristic of the plant origin, therefore rice starch granules can be differentiated from starch granules from other cereals.

With regard to the presence of proteins, the Examiner's attention is drawn to the content of document D7, allegedly novelty-destroying, especially on page 7, legend of figure 15 and on page 38. In these sections, an SDS-PAGE electrophoresis of starch granule proteins is reported, thus confirming the presence of proteins in starch granules.

Moreover, Applicant also refers to page 2 of the present application, in the first lines, where it is disclosed that GBSS (granule-bound starch synthase) is a protein which is exclusively localised **within** the starch granule. This protein is embedded in the starch of the granule. Moreover, the sequence of said protein is specific for rice and different in other cereal, e.g. wheat, barley or maize. Therefore, by disrupting a starch granule and analysing the sequence of the GBSS protein found in association with the starch, it can be ascertained without any ambiguity whether this starch granule originates from rice or from another plant.

As further evidence of this point, we enclose a Declaration by Sadequr Rahman, one of the inventors of this application, confirming that the sequences of the proteins comprised in the starch granules allow the clear identification of the origin of the granule, and that the same applies to flour. These facts are unchanged with respect to high-amylose starch granules.

Therefore, the qualifying term "rice" with respect to starch granule or flour, is a functional feature which ensures the distinction between the claimed subject-matter and high-amylose starch granules or flour obtained from another plant grains.

The claimed subject-matter according to amended claims 14-17 (corresponding to previous claims 15-18) is thus novel over the cited prior art, especially disclosure of documents D6 and D7.

## III- Inventive step (Article 56 EPC):

It is noted that inventive step has been acknowledged for the claims specifically and unambiguously directed to the generation of a rice grain wherein the proportion of amylose in the starch determined by an iodometric method is at least 40%.

With regard to previous claims 31 and 32, they were objected to under article 56 EPC in the light of the disclosure of document D4. In order to expedite the prosecution of this application



and without conceding the correctness of the Examiner's objection, these claims have been deleted from the enclosed set of claims, rendering the objection now moot.

With regard to previous independent claims 34 and 36 (now numbered 28 and 30), a reference to high amylose content has been introduced into these claims. Support for such an amendment can be found in the application as filed in the last paragraph of page 12, inter alia at lines 19-20 and 31-36.

In view of the amendments introduced into the set of claims, all the independent claims are now clearly directed to the generation of a rice grain wherein the proportion of amylose in the starch determined by an iodometric method, is at least 40%., for which inventive step has been acknowledged.

The amended set of claims therefore fulfils the requirements of Article 56 EPC.

# IV- Clarity (Article 84 EPC)

#### IV.1, Clarity of Claim 1:

Claim 1 of the previous set of claims has been considered as unclear due to the alleged lack of technical features and due to the absence of reference accompanying the term "reduced".

With respect to the first of these points, without acknowledging the correctness of the Examiner's objection, it is submitted that claim 1 has been amended in order to incorporate the specific technical features previously recited into claim 2.

It is now clearly specified in amended claim 1 that the reduced levels of SBEIIa and SBEIIb proteins and/or activities are obtained further to a genetic mutation in the genes coding for these proteins or further to the introduction of a nucleic acid, which inhibits the expression and/or activity of said two proteins.

Both genetic variations are perfectly clear for a skilled person; indeed, genes coding for both proteins are well-known and their wild-type sequences are known from publicly available databases. Moreover, the genetic variation consisting in the introduction of a nucleic acid is also a known and routine technique, this is thus perfectly clear to the skilled man, what such a genetic variation covers.

Finally, means for measuring the level of expression or activity of said proteins are well known to the skilled persons and some of them are mentioned in the paragraph bridging pages 13 and 14.

Therefore, it is concluded that the technical features now recited into amended claim 1 are perfectly clear and define the claimed subject-matter in a concrete manner, and not allegedly by mention of a result to be achieved.

Applicant is also of the opinion that a more restrictive definition of the subject-matter would be an undue limitation with respect to the technical contribution of the present invention. The inventors have indeed obtained for the first time rice grain with a very high proportion of amylose, namely at least 40%. They have also elucidated the mechanism allowing this high proportion of amylose in the rice to be obtained, that is they have defined a combination of two proteins, whose expression is to be decreased at least in the endosperm, to obtain such a rice starch. Once the combination of proteins has been identified, different means are known to decrease their level of expression and/or activity and limiting the scope of the claims to a unique embodiment would be an undue limitation.

With respect to the second point raised against the clarity of claim 1, it is to be noted that this claim has been amended in order to recite that the decreased levels in the claimed grain are with respect to wild type rice grain. Support for such an amendment can be found *inter alia* in the first paragraph on page 13, or in the last paragraph on page 12 of the application as filed.

Moreover, the mention that decreased or increased levels are with reference to wild-type rice, has been incorporated into all the claims where such an expression was present.

Further to the modifications introduced into claim 1, Applicant is of the opinion that amended claim 1 according to the enclosed set of claims fulfils the requirements of article 84 EPC.

## IV.2. Clarity of the term "altered":

Previous claim 8 has been objected to under article 84 EPC, due to the term "altered" which is found unclear, especially in the absence of a reference level.

Without acknowledging the correctness of this objection, it is submitted that this claim has been amended in order to replace the term "altered" by the terms "reduced or increased", and to add a reference to levels in wild-type rice.

Support for such an amendment can be found in the application as filed in the second paragraph on page 14 and in the first paragraph on page 13.

Further to these amendment, previous claim 8, corresponding to current 7, is to be considered as fulfilling the requirements of article 84 EPC.

#### IV.3. Clarity of the term "non-shrunken":

The Examiner has reiterated his objection against previous claim 10, due to the recitation of the feature "non-shrunken".

Applicant maintains that this term is a usual one in the filed of the invention. However, in order to expedite the prosecution of this application, the definition of this term given in the description, has been added into the claim, i.e. that a non-shrunken grain shows a plump or fully-filled phenotype. This definition is to be found in the application as filed on page 19, lines 3-4.

In view of this amendment, the lack of clarity objection against previous claim 10, corresponding to amended claim 9, is thus overcome.

# IV.4. Clarity of the claims to rice plant:

Previous claim 14 has been objected to by the Examiner as allegedly lack any technical feature.

Without conceding the correctness of this objection, it is submitted that present claim 13, corresponding to previous claim 14, has been amended to incorporate the features now also recited into amended claim 1.

Support for such an amendment can be found in the application as filed *inter alia* on the last paragraph on page 12.

It has been detailed in the section dealing with clarity of claim, why the incorporation of these features is considered to overcome the Examiner's objection.

In view of the foregoing, amended claim 13 is in keeping with the requirements of article 84 EPC.

#### IV.5. Clarity of the claims to rice starch granules and flour:

Previous claim 18 has been objected to by the Examiner for clarity reasons as he is of the opinion that the reference to the rice origin of the starch granule cannot be considered as a valid technical feature.

Contrary to the Examiner's assertion, a rice starch granule can be easily distinguished from another starch granule, for example a wheat or maize starch granule. Indeed, the shape and size of starch granules differ between species, as does also the carbohydrate distribution,



namely lengths of the chains. Therefore, starch granules from different plants can be distinguished visually or by very simple assays.

Moreover, as detailed in the section dealing with novelty, starch granules contain, in addition to starch, <u>some proteins and lipids</u> which are characteristic of the plant origin, allowing distinction between starch granules from rice and from other cereals.

Attention is drawn to the enclosed Declaration by Sadequr Rahman, as further evidence of the ability to distinguish rice starch granules from other plant starch granules, for example on the basis of sequence specificity of the proteins embedded in the granules. Moreover, it is also stated in this Declaration that the same applies to flour, which also comprises proteins characteristic of the plant origin of the starch.

Therefore, the term "rice" with respect to starch granule or flour, is a valid functional feature which ensures the distinction between the claimed subject-matter and high-amylose starch granules or flour obtained from another plant grains.

Moreover, the present inventors were the first ones to obtain rice grains with a proportion of amylose in the starch of at least 40%. They obtained such rice grains by decreasing the activity of both SBEIIa and SBEIIb proteins. At the time of the invention, there is no other means to obtain such grains, and thus no other means to obtain high-amylose rice starch granules. Therefore there is no other rice starch granule with a proportion of amylose in the starch of at least 40% in the prior art from which the rice starch granules of the invention should differentiate themselves. The same applies mutatis mutandis to rice flour.

In view of these remarks, amended claims 14-17, corresponding to previous claims 15-18, are to be considered as fulfilling the requirements of clarity.

#### IV.6. Clarity of the method according to claim 19:

Previous claim 19 has been objected due to the reference to a genetic variation without mention of its nature.

In view of this objection, please note that this claim, now numbered 18, has been amended in order to incorporate the features previously recited in claim 20, i.e. two genetic variations are to be introduced which are either genetic mutations into the genes coding for SBEIIa or SBEIIb protein, or nucleic acids, which inhibit the expression and/or activity of said two proteins.

For the reasons detailed in the section dealing with clarity of claim 1, it is considered that the genetic variations to be introduced are defined in such a way that a skilled person perfectly appreciates the type of variations covered by this claim.



The same applies to the dependent claims 19-26.

With respect to the claims previously numbered 31 and 32, they have been deleted from the enclosed set of claims. The objection in connection with these claims is thus now moot.

# IV.7. Reference to mutagenesis:

Previous claim 25 has been objected to due to the reference to mutagenesis in this claim. Without conceding the correctness of this objection, it is submitted that previous claim 25 has been deleted from the enclosed set of claims and no other claim refers to thus subject-matter.

The objection in connection with this claim is thus now without object.

# IV.8. Clarity of the term "altered":

Previous claim 33 has been objected to due to the recitation of "altered rice starch" in this claim. This claim, now numbered 27, has been amended in order to replace the term "altered" by a reference to a proportion of amylose of at least 40%. Support for such an amendment can be found in the application as filed, on page 12, lines 19-21.

In view of the foregoing, amended claim 27 is to be considered as fulfilling the requirements of article 84 EPC.

#### IV.9. Clarity of the term "co-suppression":

Previous claim 36 has been objected to due to the recitation of "co-suppresion" inhibitor.

Without conceding the correctness of this objection, the claim, which is now numbered 30 has been amended in order to delete the objected term. Further to this modification, it is submitted that this claim should now be considered as complying with article 84 EPC.

The same conclusion is to be applied to the dependent claims 31-34.

#### V- Conclusions:

It is believed that claims 1 to 34 according to the enclosed set of claims comply with all the requirements of the EPC.

Should any issues remain outstanding, the undersigned would be pleased to discuss the case by telephone with the Examiner.

Should the Examining Division intend to refuse this application, Oral Proceedings are requested as a precautionary measure under Art. 116 EPC.

Yours sincerely,

Carol SELLIN

Professional Representative before the European Patent Office

Encl.: Acknowledgment of receipt (Form 1037.1 EPO)

Amended set of claims (clean and marked-up copies)

Declaration by Sadequr Rahman Annex A: table detailing support

# ANNEX A:

# SUPPORT FOR THE AMENDED SET OF CLAIMS IN THE APPLICATION AS FILED

Amended claims	Claims previously on file	Support for the amendments
1	1-2	Page 13, lines 1-5
2-5	3-6	No change apart renumbering and adaptation of claim dependency
6	7	Page 13, lines 1-5
7	8	Page 14, lines 15-24 and page 13, lines 1-5
8	9	Page 13, lines 1-5 and page 31, lines 1-3
9	10	Page 19, lines 1-4
10-12	11-13	No change apart renumbering and adaptation of claim dependency
13	14	Page 12, lines 19-36
14-17	15-18	No change apart renumbering and adaptation of dependency
18	19-20	Page 13, lines 1-5
19-23	21-24 and 26	No change apart renumbering and adaptation of claim dependency
	25	deleted
24	27	Page 13, lines 1-5
	28	deleted
25-26	29-30	No change apart renumbering and adaptation of claim dependency
	31-32	deleted
27	33	page 12, lines 19-21
28	34	last paragraph of page 12, inter alia at lines 19-20 and 31-36 and page 13, lines 1-5
29	35	No change apart renumbering and adaptation of claim dependency
30	36	last paragraph of page 12, inter alia at lines 19-20 and 31-36
31-34	37-40	No change apart renumbering and adaptation of claim dependency

# **CLAIMS**

- 1. Grain obtained from a rice plant, comprising starch, wherein the proportion of amylose in the starch of the grain determined by an iodometric method is at least 40%, said grain comprising reduced levels of SBEIIa and SBEIIb proteins and/or activities with respect to wild-type rice grain, and wherein said grain comprises two or more genetic variations, wherein one genetic variation is selected from the group consisting of
  - a) a mutation of an SBEIIa gene which inhibits SBEIIa expression and/or activity, and
- b) an introduced nucleic acid which inhibits SBEIIa expression and/or activity, and and wherein a second genetic variation is selected from the group consisting of
  - c) a mutation of an SBEIIb gene which inhibits SBEIIb expression and/or activity, and
  - d) an introduced nucleic acid which inhibits SBEIIb expression and/or activity.
- 2. The grain of claim 1, wherein the proportion of amylose in the starch of the grain is at least 50%.
- 3. The grain of any one of claims 1 to 2, wherein the reduced levels of SBEIIa and SBEIIb proteins and/or activities are obtained by introduction of a transgene into the rice plant.
- 4. The grain of claim 3, wherein the transgene encodes an antisense, co-suppression, ribozyme or duplex RNA molecule.
- 5. The grain of any one of claims 1 to 2 which is non-transgenic.
- 6. The grain of any one of claims 1 to 5, further comprising a reduced level of SBEI protein and/or activity with respect to wild-type rice grain.
- 7. The grain of any one of claims 1 to 6, comprising an increased or decreased level of an enzyme activity by at least 40% compared to the activity in unmodified rice, wherein

said enzyme is selected from the group consisting of ADP glucose pyrophosphorylase, GBSS, SSI, SSII, SSIII, a debranching enzyme of an isoamylase type and a debranching

enzyme of a pullulanase type.

8. The grain of claim 1, comprising an increased level of GBSS protein with respect to

wild-type rice.

9. The grain of any one of claims 1 to 8 which is non-shrunken in that it shows a

plump or fully-filled phenotype.

10. The grain of any one of claims 1 to 9 which is brown rice having an average weight

of at least about 25 mg.

11. The grain of any one of claims 1 to 10, comprising a null mutation of the SBEIIa or

SBEIIb gene.

12. The grain of any one of claims 1 to 11 which is of an Indica variety or which

comprises a Wx<sup>a</sup> allele.

13. A rice plant capable of producing the grain according to any one of claims 1 to 12,

wherein said rice plant comprises two or more genetic variations, wherein one genetic

variation is selected from the group consisting of

a) a mutation of an SBEIIa gene which inhibits SBEIIa expression and/or activity in

the endosperm, and

b) an introduced nucleic acid which inhibits SBEIIa expression and/or activity in the

endosperm, and

and wherein a second genetic variation is selected from the group consisting of

c) a mutation of an SBEIIb gene which inhibits SBEIIb expression and/or activity in

the endosperm, and

d) an introduced nucleic acid which inhibits SBEIIb expression and/or activity in the

endosperm.

- Rice starch granules extracted from the grain according to any one of claims 1 to 12 wherein the proportion of amylose in the starch determined by an iodometric method is at least 40%.
- 15. A product comprising rice flour produced from the grain according to any one of claims 1 to 12, said flour comprising starch, wherein the proportion of amylose in the starch, determined by an iodometric method, is at least 40%.
- 16. The product of claim 15 wherein the flour is blended with flour or starch from another source.
- 17. The product of claim 15 which is a non-food product.
- 18. A method of producing a rice plant capable of producing grain, the grain having starch comprising at least 40% amylose, as determined by an iodometric method, comprising the steps of
  - a) introducing two or more genetic variations into a parent rice plant or seed; and
  - b) identifying a progeny plant of the parent rice plant or seed, wherein the starch of grain of the progeny plant comprises at least 40% amylose, as determined by an iodometric method,

wherein the genetic variations lead to a reduction of the levels of SBEIIa and SBEIIb proteins and/or activities in the endosperm of the rice plant with respect to wild-type rice plant and wherein one genetic variation is selected from the group consisting of

- (i) a mutation of an SBEIIa gene which inhibits SBEIIa expression and/or activity, and
- (ii) an introduced nucleic acid which inhibits SBEIIa expression and/or activity, and

and wherein a second genetic variation is selected from the group consisting of

- (iii) a mutation of an SBEIIb gene which inhibits SBEIIb expression and/or activity, and
- (iv) an introduced nucleic acid which inhibits SBEIIb expression and/or activity.

The method of claim 18 wherein the step of introducing the genetic variation 19.

comprises introducing an exogenous nucleic acid.

The method of claim 19 wherein the exogenous nucleic acid is introduced into a 20.

rice cell which is then regenerated into a rice plant.

21. The method of claim 20 wherein the exogenous nucleic acid encodes an inhibitor of

SBEIIa and/or SBEIIb expression and/or activity.

22. The method of claim 21 wherein the inhibitor is an antisense, co-suppression,

ribozyme or duplex RNA molecule.

23. The method of any one of claims 18 to 22, wherein the progeny rice plant

comprises a null mutation in SBEIIa and/or SBEIIb.

24. The method of any one of claims 18 to 23 which further comprises the step of

introducing a genetic variation which leads to a reduction in the level of SBEI protein

and/or activity with respect to wild-type rice plant.

25. The method of any one of claims 18 to 24 which further comprises the introduction

of a  $Wx^a$  allele into the rice plant.

The method of claim 25 wherein the  $Wx^a$  allele is introduced by crossing. 26.

27. A method of producing rice starch having a proportion of amylose determined by

an iodometric method of at least 40%, comprising the step of extracting starch from the

grain according to any one of claims 1 to 12.

28. Use of two or more exogenous nucleic acid molecules, at least one of which

encodes an inhibitor of rice SBEIIa expression and/or activity and at least another of which

encodes an inhibitor of rice SBEIIb expression and/or activity, to produce a rice plant

EP04789648.5 - 2405 February 2009

which has reduced levels of SBEIIa and SBEIIb proteins and/or activities with respect to

wild-type rice plant and a proportion of amylose in the starch of the grain determined by an

iodometric method of at least 40%.

29. The use of claim 28 wherein the inhibitors are selected from the group consisting of

antisense molecules, ribozymes, duplex RNA molecules and any combination of these.

30. An isolated nucleic acid molecule which encodes an inhibitor of rice SBEIIa and an

inhibitor of rice SBEIIb, which may be the same or different, wherein said inhibitor is an

antisense, ribozyme or duplex RNA molecule capable of increasing the relative proportion

of amylose in the starch of a rice grain, determined by an iodometric method, to at least

40%.

31. A vector which comprises the isolated nucleic acid molecule of claim 30.

32. A cell which comprises the isolated nucleic acid molecule of claim 30.

33. The cell of claim 32 which is a rice cell.

34. A transgenic rice plant comprising the isolated nucleic acid molecule of claim 30.

EP04789648.5 - 2405 Amended set of claims

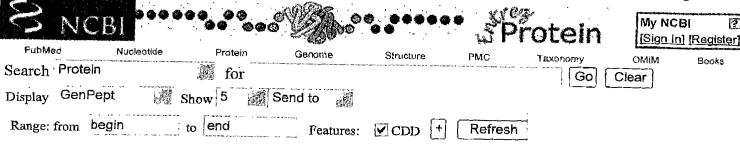
#### Declaration of Sadequr RAHMAN

- I, Sadequr Rahman, hereby declare as follows:
- I am employed by Commonwealth Scientific and Industrial Research Organisation (C.S.I.R.O.)
  as Principal Research Scientist. I am co-inventor of the European Patent Application
  EP04 789 648.5 in the name of Commonwealth Scientific and Industrial Research Organisation,
  deriving from the PCT application PCT/AU2004/001517 filled on 27<sup>th</sup> October 2004.
- 2. As co-inventor of European patent application EP04 789 648.5, I am familiar with the content of this application. I am aware that in the course of Examination proceedings, a question has arisen with respect to the distinction between starch granules from rice and from other cereals.
- 3. I confirm that starch granules from cereals contain starch, together with some proteins and lipids. This is certainly true for rice starch granules. Amongst these proteins is the granule-bound starch synthetase (GBSS). This protein is embedded in the starch granule and tightly bound to the starch. To the best of my knowledge, there is no way to obtain starch devoid of GBSS from rice starch granule without completely disrupting the granule.
- 4. I also confirm that the amino acid sequence of the protein named granule-bound starch synthetase (GBSS) is genus-specific in plants. I refer in this respect to the GeneBank accession numbers CAA37732 for rice (Exhibit 1), AAM74048 for barley (Exhibit 2) and CAA40509 for wheat (Exhibit 3), as an illustration. Therefore, by determining the amino acid sequence of the GBSS protein embedded in a starch granule, the origin of the starch granule can be determined unambiguously. Likewise, an indication of the origin of a starch granule, as 'rice', implies that the associated GBSS protein will be specific to rice.
- 5. Furthermore, I confirm that proteins are also comprised in rice flour. Therefore, the above-mentioned specificities of rice starch granules also apply to rice flour.

- Moreover, I also confirm that the sequence specificity mentioned above also applies to cereals
  producing starch with a higher amylose content in starch than the corresponding wild type
  cereal.
- 7. I am aware that this Declaration and accompanying Exhibits are to be submitted to the European Patent Office as part of the response to the Official Communication dated 29<sup>th</sup> July 2008.
- 8. All statements made herein of my own knowledge are true and all statements made on information are believed to be true.

8th Feb, 2009.

Sadegm Palma Signature



EP04 789 648.5

Exhibit 1

 $\square$ 1: CAA37732. Reports starch synthase [...[gi:577599]

Features Sequence

LOCUS CAA37732 609 aa linear PLN 14-NOV-2006

DEFINITION starch synthase [Oryza sativa (japonica cultivar-group)].

ACCESSION CAA37732

VERSION CAA37732.1 GI:577599
DBSOURCE embl accession X53694.1

KEYWORDS

SOURCE Oryza sativa (rice)

ORGANISM Oryza sativa

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BEP

clade; Ehrhartoideae; Oryzeae; Oryza.

REFERENCE 1 (residues 1 to 609)

AUTHORS Wang, Z.Y., Wu, Z.L., Xing, Y.Y., Zheng, F.G., Guo, X.L., Zhang, W.G. and

Hong, M.M.

TITLE Nucleotide sequence of rice waxy gene JOURNAL Nucleic Acids Res. 18 (19), 5898 (1990)

PUBMED 2216792

REFERENCE 2 (residues 1 to 609)

AUTHORS Wang, Z.Y.

TITLE Direct Submission

JOURNAL Submitted (12-JUN-1990) Wang Z., Shanghai Institute of Plant

Physiology, Academia Sinica, 300 Fenglin Road, Shanghai 200032,

China

FEATURES Location/Qualifiers

source 1..609

/organism="Oryza sativa"
/cultivar="Hanfeng"
/db\_xref="taxon:4530"

/clone="lambda-Wx2 and lambda-Wx5"

/clone\_lib="lambda EMBL3"

Protein 1..609

/product="starch synthase"

<u>Region</u> 83..579

/region name="glgA"

/note="glycogen synthase; Provisional; PRK00654"

/db\_xref="CDD:100628"

Region 84..581

/region\_name="GT1\_Glycogen\_synthase\_DULL1\_like"

/note="This family is most closely related to the GT1

family of glycosyltransferases. Glycogen synthase

catalyzes the formation and elongation of the

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cd03791"

/db xref="CDD:99965"

<u>Site</u> order(97,100,406..408,462..463,468,485,490)

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/note="ADP-binding pocket"

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<u>Site</u> order(327,505,510..511,513,542)

/site\_type="other"

/note="homodimer interface"

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                     X53694.1:2580..2689,X53694.1:2811..3054,
                     X53694.1:3172..3348, X53694.1:3592..3783,
                     X53694.1:3890..3976, X53694.1:4086..4214,
                     X53694.1:4572..4688)"
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     121 vispryddyk dawdtsvvae ikvadryerv rffhcykrgv drvfidhpsf lekvwgktge
      181 kiygpdtgvd ykdnqmrfsl lcqaaleapr ilnlnnnpyf kgtygedvvf vcndwhtgpl
     241 asylknnyqp ngiyrnakva fcihnisyqg rfafedypel nlserfrssf dfidgydtpv
     301 egrkinwmka gileadrvlt vspyyaeeli sgiargceld nimrltgitg ivngmdvsew
     361 dpskdkyita kydattaiea kalnkealqa eaglpvdrki pliafigrle eqkgpdvmaa
      421 aipelmqedv qivllgtgkk kfekllksme ekypgkvrav vkfnaplahl imagadvlav
      481 psrfepcgli qlqgmrygtp cacastgglv dtviegktgf hmgrlsvdck vvepsdvkkv
     541 aatlkraikv vgtpayeemv rncmnqdlsw kgpaknwenv llglgvagsa pgiegdeiap
      601 lakenvaap
11
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Exhibit 2

☐ 1: AAM74048. Reports granule bound sta...[gi:21667428]

#### Features Sequence

LOCUS 603 aa AAM74048 linear PLN 12-SEP-2002

DEFINITION granule bound starch synthase I [Hordeum vulgare].

ACCESSION AAM74048

AAM74048.1 GI:21667428 VERSION DBSOURCE accession AF486514.1

KEYWORDS

COMMENT

SOURCE Hordeum vulgare ORGANISM Hordeum vulgare

> Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BEP

clade; Pooideae; Triticeae; Hordeum.

(residues 1 to 603) REFERENCE 1

**AUTHORS** Patron, N.J., Smith, A.M., Fahy, B.F., Hylton, C.M., Naldrett, M.J.,

Rossnagel, B.G. and Denyer, K. TITLE The Altered Pattern of Amylose Accumulation in the Endosperm of

> Low-Amylose Barley Cultivars Is Attributable to a Single Mutant Allele of Granule-Bound Starch Synthase I with a Deletion in the

5'-Non-Coding Region

JOURNAL Plant Physiol. 130 (1), 190-198 (2002)

PUBMED 12226499

(residues 1 to 603) REFERENCE

Patron, N. AUTHORS

Direct Submission TITLE

JOURNAL Submitted (22-FEB-2002) Metabolic Biology, John Innes Centre,

Norwich Science Park, Norwich, Norfolk NR4 7UH, UK Method: conceptual translation supplied by author.

Location/Qualifiers FEATURES

> 1..603 source

> > /organism="Hordeum vulgare" /cultivar="Oderbrucker" /db xref="taxon:4513"

1..603 Protein

/product="granule bound starch synthase I"

/name="alpha glycosyl transferase"

76..573 Region

/region\_name="glgA"

/note="glycogen synthase; Provisional; PRK00654"

/db\_xref="CDD: 100628"

77..575 Region

/region\_name="GT1\_Glycogen\_synthase\_DULL1\_like"

/note="This family is most closely related to the GT1 family of glycosyltransferases. Glycogen synthase

catalyzes the formation and elongation of the

alpha-1,4-qlucose backbone using ADP-glucose, the second and key step of glycogen biosynthesis. This family ...;

cd03791"

/db\_xref="CDD:99965"

Site order (90, 93, 399..401, 456..457, 462, 479, 484)

/site\_type="other"

/note="ADP-binding pocket"

/db\_xref="CDD:99965"

<u>Site</u> order(320,499,504..505,507,536)

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/site type="other"
                     /note="homodimer interface"
                     /db_xref="CDD:99965"
     CDS
                     1..603
                     /coded by="AF486514.1:1..1812"
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      121 qykdawdtsv iseikvadey ervrffhcyk rgvdrvfidh pwflekvrgk tkekiygpda
      181 gtdyedngqr fsllcqaale apriln1nnn pyfsgpyged vvfvcndwht gllacylksn
      241 yqsngiyrta kvafcihnis yqgrfsfddf aqlnlpdrfk ssfdfidgyd kpvegrkinw
      301 mkagilqadk vltvspyyae elisgeargc eldnimrltg itgivngmdv sewdptkdkf
      361 lavnyditta leakalnkea lqaevglpvd rkvplvafig rleeqkgpdv miaaipeilk
      421 eedvqiillg tgkkkfekll ksmeekfpgk vravvrfnap lahqmmagad llavtsrfep
      481 cgliqlqgmr ygtpcvcast gglvdtiveg ktgfhmgrls vdcnvvepad vkkvattlkr
      541 avkvvgtpay qemvkncmiq dlswkgpakn wedvllelgv egsepgivge eiaplamenv
      601 aap
11
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Exhibit 3

1: CAA40509. Reports glycogen (starch)...[gi:21902]

Features Sequence

LOCUS CAA40509 615 aa linear PLN 09-SEP-2004

DEFINITION glycogen (starch) synthase [Triticum aestivum].

ACCESSION CAA40509

VERSION CAA40509.1 GI:21902 DBSOURCE embl accession X57233.1

KEYWORDS .

SOURCE Triticum aestivum (bread wheat)

ORGANISM Triticum aestivum

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BEP

clade; Pooideae; Triticeae; Triticum.

REFERENCE 1 (residues 1 to 615)

AUTHORS Clark, J.R., Robertson, M. and Ainsworth, C.C.

TITLE Nucleotide sequence of a wheat (Triticum aestivum L.) cDNA clone

encoding the waxy protein

JOURNAL Plant Mol. Biol. 16 (6), 1099-1101 (1991)

PUBMED 1863765

REMARK Erratum: [Plant Mol Biol 1991 Oct; 17(4):957]

REFERENCE 2 (residues 1 to 615)

AUTHORS Clark, J.R.

TITLE Direct Submission

JOURNAL Submitted (04-DEC-1990) J.R. Clark, WYE COLLEGE, UNIVERSITY OF

LONDON, DEPT OF BIOCHEMISTRY & BIOLOGICAL SC., NR. ASHFORD KENT

TN25 5AH, U K

FEATURES Location/Qualifiers

source 1..615

/organism="Triticum aestivum"

/db\_xref="taxon:4565"
/chromosome="7A, 7B, 4B"

/clone="pcss22"

/cell\_line="Chinese spring"
/tissue\_type="endosperm"
/clone lib="lambda gt10"

Protein 1..615

/product="glycogen (starch) synthase"

/EC number="2.4.1.11"

<u>Region</u> 77..585

/region\_name="glgA"

/note="glycogen synthase; Provisional; PRK00654"

/db xref="CDD:100628"

<u>Region</u> 78..587

/region\_name="GT1\_Glycogen\_synthase\_DULL1\_like"

/note="This family is most closely related to the GT1

family of glycosyltransferases. Glycogen synthase

catalyzes the formation and elongation of the

alpha-1,4-glucose backbone using ADP-glucose, the second and key step of glycogen biosynthesis. This family...;

cd03791"

/db xref="CDD:99965"

Site order(91,94,411..413,468..469,474,491,496)

/site\_type="other"

/note="ADP-binding pocket"

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                     /coded by="X57233.1:64..1911"
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                     /db xref="InterPro:IPR011835"
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       61 drrclsmvvr atgsggmnlv fvgaemapws ktgglgdvlg glpaamaang hrvmvispry
      121 dqykdawdts viseikvvdr yervryfhcy krgvdrvfvd hpcflekvrg ktkekiygpd
      181 agtdyednqq rfsllcqaal evprildlnn nphfsgpyam lcravprrag edvvfvcndw
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      361 dvsewdpikd kfltvnydvt talegkalnk ealqaevglp vdrkvplvaf igrleegkgp
      421 dvmiaaipei vkeedvqivl lgtgkkkfer llksveekfp tkvravvrfn aplahqmmag
      481 advlavtsrf epcgliqlqg mrygtpcaca stgglvdtiv egktgfhmgr lsvdcnvvep
      541 advkkvvttl kravkvvgtp ayhemvkncm iqdlswkgpa knwedvllel gvegsepgiv
      601 geeiaplale nvaap
//
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